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ORIGINAL ARTICLE

Resistant starch content of conventionally boiled and pressure-cooked cereals, legumes and tubers

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Abstract Resistant starch (RS) content was determined in the conventionally boiled (H_1) and pressure-cooked (H_2) cereals, legumes and tubers using enzymatic method. Both H_1 and H_2 legumes contained higher amount of RS as compared to cereals and tubers. H_1 and H_2 lentils showed highest RS content of 5.0 and 4.9% (dwb), respectively. Higher RS content in legumes can be attributed to the presence of intact tissue/cell structures enclosing starch granules and high level of amylose (26–33%) and high content of viscous soluble dietary fiber components. The decrease in RS content of H_2 foods in comparison to H_1 counterparts (maximum decrease of 15% in pea) might have occurred due to changes in cell wall integrity of H_2 foods and this could result in increased accessibility of starch to amylolytic enzymes.

Keywords Cereals · Legumes · Tubers · Resistant starch · Amylose content · Conventional boiling · Pressure-cooking

Introduction

Food starches may be termed as either glycemic or resistant (Berry 1986, Englyst et al. 1992). Resistant starch (RS) is not hydrolyzed even after 120 min of incubation, however, it is fermented by gut microflora (Englyst et al. 1992). RS includes starch and starch degradation products, which escape enzymatic hydrolysis in the small intestine and get fermented in the colon. RS has been categorized as RS_1, RS_2, RS_3 and RS_4. RS_1 represents tightly bound starch molecules wrapped in fiber shell that is physically inaccessible to digestive enzymes (Bird et al. 2000, Haralampu 2000) and is found in partly milled grains and seeds. RS_2 is ungelatinized starch granule, which is unavailable to amylolytic enzymes due to its compact and unhydrated structure. RS_3 is the retrograded or recrystallized starch and is found in most of the heat processed and cooled foods (Muir and O'Dea 1992, Haralampu 2000). RS_4 is the chemically modified form, for example, esterified and cross-bonded starch that cannot be broken down, since the modification process renders the structure inaccessible to digestion by α-amylase (Haralampu 1998).

RS has assumed importance due its various beneficial health properties mostly mediated by short chain fatty acids produced during its fermentation in the large intestine (Premavalli et al. 2006, Sharma et al. 2008). RS may be either present naturally in foods or may be generated during various processing/cooking or storage conditions. Very little raw starch is consumed in normal diets and most of the processed foods invariably involve the application of heat and moisture for varying periods. The RS being a promising ingredient in processed foods, it is worth to examine the RS content in various foods. Few studies are available on the RS content of Indian foods (Kavita et al. 1998, Mahmood et al. 2006, Katyal et al. 2005, Mahadevamma and Tharanathan 2004). Conventional boiling (H_1) and pressure cooking (H_2) are the common household and industrial methods of cooking foods. Since, the extent and severity of the heating during 2 cooking methods are different, the extent of gelatinization and retrogadation of starch, which govern the formation of RS also differs. Therefore, the present study was undertaken to study RS content of H_1 and H_2 cereals, legumes and tubers commonly consumed in India.

Materials and methods

Seeds of Bengal gram (*Cicer arietinum*), pea (*Pisum sativum*), lentil (*Lens esculenta*), kidney beans (*Phaseolus vulgaris*), grains of wheat (*Triticum aestivum*), rice (*Oryza*

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sativa), barley (*Hordeum vulgare*) and tubers of potato (*Solanum tuberosum*) and sweet potato (*Ipomoea batatas*) were procured from local market of Hisar (2 kg each). The legume grains were milled to *dhals* in the locally available *dhal* mill. The flours from various legume and cereal crops were prepared by using Navdeep flourmill available in the Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar.

All chemicals used were of analytical grade. The enzymes used for analytical purposes were pepsin (Merck No. 7190, 2002 FIP U/G, Darmstadt, Germany), pancreatic alpha amylase (Sigma, A-3176, Saint Louis, MO, USA), amyloglucosidase from *Aspergillus niger* (Fluka, 10115, Buchs, Switzerland), glucose oxidase from *Aspergillus niger* (SRL 074040, Mumbai, India), peroxidase from horseradish (Himedia RM 664, Mumbai, India), heat-stable alpha amylase (Sigma, A-3306, Saint Louis, MO, USA) and protease (Sigma, P 3910, Saint Louis, MO, USA).

The raw cereals, legumes and tubers were analyzed for their moisture, ash, fat and protein contents by employing AOAC (1984) methods. Amylose content was determined by using the rapid colorimetric method of Williams et al. (1970). Total starch content was determined as the glucose released by the enzymic hydrolysis after gelatinization of samples in boiling water Goni et al. (1997). The dietary fiber contents were determined by method of AOAC (1995) except no corrections were made for protein and ash in the undigested residue. Dried and finely ground sample (500 mg) was taken in a 50 ml screw cap tube and dispersed with 25 ml of 0.1M-phosphate buffer (pH 6.5); 50 µ*l* of heat-stable alpha amylase was added and incubated in a boiling water bath for 30 min with shaking at 5 min intervals. The contents were cooled to room temperature (32°C), pH was adjusted to 7.5 with 0.5 M NaOH solution, protease solution (100 µl*)* was added and incubated for 30 min at 60°C with continuous agitation. The pH was adjusted to 4.5 with 0.56 M HCl and the contents were incubated with 300 µl of amyloglucosidase for 30 min at 60°C with constant shaking. The contents were centrifuged at 3000xg for 20 min. The insoluble residue was washed with 78% ethanol (15 ml, 2x), absolute alcohol and acetone. The residue was dried at 105°C for 5 h in hot air oven. The dried residue was weighed and insoluble dietary fiber (IDF) was expressed as percentage of dry matter. For determination of soluble dietary fiber (SDF), the supernatant was added with 4 volumes of 95% ethanol (about 200 ml, preheated to 60°C) and the mixture was allowed to stand for 1 h at room temperature followed by centrifugation (3000x**g**) and weighing the residue. Sum of IDF and SDF was reported as total dietary fiber (TDF).

RS content was measured in freshly boiled/cooked samples using enzymatic method of Goni et al. (1996). 400–500 mg of wet homogenized sample was weighed in a 50 ml centrifuge tube. Pepsin solution (0.2 ml, 1 g pepsin/10 ml of KCl-HCl buffer, pH 1.5) was added to deproteinize the sample. After mixing and shaking the tubes in a water bath shaker at 40°C for 60 min, the sample was cooled to room temperature. The sample was treated with pancreatic alpha amylase (1 ml, 40 mg alpha-amylase per ml of Tris maleate buffer), mixed and incubated for 16 h in a water bath at 37°C with constant shaking to hydrolyze digestible starch. Centrifugation of the sample (15 min, 3000xg) was carried out and the supernatant discarded. The pellet was washed with distilled water and centrifuged again to discard supernatant. Distilled water (3 ml) and 3 ml of 4M KOH were added, mixed with a magnetic stirrer and allowed to shake constantly for 30 min at room temperature. After the complete dispersion of sample, 5.5 ml of 2 m HCl and 3 ml of 0.4 M sodium acetate buffer (pH 4.75, pH adjusted with 2 M HCl) and 80 µl of amyloglucosidase (5 mg/ml of acetate buffer pH 4.75) were added and placed in a water bath at 60°C for 45 min with constant shaking. The contents were centrifuged (15 min, 3000xg), supernatant was collected in a 500 ml volumetric flask. The residue was washed with 10 ml of distilled water, centrifuged again and the supernatant was combined with that obtained previously. The volume was made to 250–500 ml depending upon the RS content. The amount of glucose was determined by spectrophotometric method using glucose oxidase-peroxidase reagent.

RS (% of the sample as is) was calculated as

RS content, % = Glucose concentration from standard curve × 0.9 × volume correction × 1/1000 × 100/w.

Conventional boiling (H_1)*:* The finely milled rice, wheat and barley flours (60 mesh particle size) were suspended in water (10 g/50 ml) and heated in a boiling water bath for 30 min. Legume flour (5.0 g of each legume) was suspended in 20 ml of water and heated on a boiling water bath for 30 min at 70°C. The potato and sweet potato tubers were boiled intact with peel in an uncovered pan using a sample to water ratio of 1:3 (w/v) at 100°C. The time taken for boiling was 30 min for potato and 20 min for sweet potato. The excess water was drained off.

Pressure-cooking (H_2)*:* The finely milled rice, wheat and barley flours (60 mesh particle size) were suspended in water (10 g/ 50 ml of distilled water) and autoclaved at 1 kg/cm^2 pressure (121°C) for 10 min. Legume flours, potato and sweet potato were autoclaved at the same pressure for 15, 10 and 15 min, respectively using sample to water ratio of 1:2 (w/v).

Statistical methods: Data were analyzed using one-way and two-way analysis of variance (ANOVA) procedures in a randomized complete block design with 3 replications. Statistical analysis was performed using the OPSTAT software version opstat1.exe.

Results and discussion

Chemical composition of food grains and tubers: The fat contents of the grains and tubers were less than 5% and kidney bean showed maximum content of 4.6% (Table 1).

Under such low amount of lipids in foods, there is very less tendency of amylose to form complex with lipids. The tubers showed negligible amounts of protein content as compared with cereals and legumes ($p \leq 0.05$) (Table 1). Tubers, Bengal gram and pea had almost same ash contents.

Dietary fiber profile, total starch and amylose contents: The total dietary fiber (TDF) values of legumes varied from the lowest value 17.2% for kidney beans to the highest value of 24.9% for Bengal gram (Table 2). Bravo et al. (1999) also observed comparable value of TDF for Bengal gram. The SDF content of 2.9% in wheat flour was in agreement with that of Tharanathan and Tharanathan (2001). The difference between the IDF values of cereals and legumes was highly significant ($p \leq 0.05$). The SDF content of potato was, however, more when compared to cereals and legumes ($p \leq 0.05$).

The total starch content of different cereal foods varied from 65.6% for barley to 81.4% for rice (Table 2). Total starch content of rice was comparable to the value found by Rosin et al. (2002). Among legumes, Bengal gram showed the highest value of total starch content (60.3%). The total starch contents for pea and Bengal gram were comparable to those found by Rosin et al. (2002). The amylose content of legumes was generally higher than that of cereals and tubers. The amylose contents of legumes were in agreement with those observed by Rosin et al. (2002).

RS content: In cereal flours RS content varied from 1.4 to 2.8% (Table 3). H_1 and H_2 treatments had marginal effect on RS content of wheat, rice and barley. The percent decrease in RS content of H_2 legume flours over H_1 flours varied from 1.6 to 15%. The RS content in different legumes varied from a minimum of 3.4% in pea to a maximum of 4.9% in lentils. The RS contents of H_1 legumes (Table 3) were marginally higher than H_2 counterparts. The RS contents of 1.7 and 1.3% in H_2 potato and sweet potato, respectively were comparable to those reported by Mahmood et al. (2006). A marginal decrease of 6.6 and 7.0% was observed in H_2 potato and sweet potato, respectively compared to H_1 tubers (Table 3). Mahmood et al. (2006) also observed no difference in RS content of H_1 and H_2 potato and sweet potato.

The RS content of legumes in general, was higher than that of cereals and tubers. The reduced bio-availability of starch and hence more RS content of legumes can be attributed to the presence of intact tissue/cell structures enclosing starch granules, high level of amylose (22–33% in our study), high content of viscous SDF components, presence of large number of antinutrients, 'B' type crystallites and strong interactions between amylose chains (Deshpande and Cheryan 1984, Hoover and Sosulski 1985, Wursch et al. 1986, Siddhuraju and Becker 2001). The starch in leguminous seeds is entrapped in parenchyma cells and swells only partially during cooking. The α-amylase cannot penetrate easily within the gelatinized starch granules due to stearic hindrance and the physical nature of leguminous starch (Wursch et al. 1986). The lower RS content of cereals

Table 1 Moisture, ash, fat and protein contents of food grains and tubers (% dwb)

	Wheat	Rice	Barley	Bengal gram	Pea	Kidney bean	Lentils	Potato	Sweet potato
Moisture	8.4 ± 0.17^{a}	11.9 ± 0.23^{b}	10.3 ± 0.20^{bc}	11.0 ± 0.35^{c}	11.2 ± 0.24^{cd}	8.1 ± 0.12^{a}	10.6 ± 0.07^{c}	77.2 ± 0.57^{e}	73.3 ± 0.58^{f}
Ash	0.9 ± 0.05^{a}	1.4 ± 0.11^{b}	0.8 ± 0.07^{a}	3.0 ± 0.10^{d}	3.7 ± 0.10^{f}	0.8 ± 0.05^{a}	0.8 ± 0.02^{a}	3.3 ± 0.13^{e}	3.1 ± 0.05^{d}
Fat	1.4 ± 0.05^{d}	0.8 ± 0.06^{c}	1.4 ± 0.10^{d}	4.6 ± 0.11^{e}	1.3 ± 0.12^{d}	1.6 ± 0.02^{d}	0.7 ± 0.09^{c}	0.2 ± 0.02^{a}	0.4 ± 0.05^{b}
Protein	12.1 ± 0.40^{c}	7.6 ± 0.37^{b}	11.4 ± 0.47^{c}	18.1 ± 0.54^{d}	20.2 ± 0.89^{e}	17.7 ± 0.77^{d}	20.7 ± 0.23^{e}	1.6 ± 0.13^{a}	1.0 ± 0.07^{a}

The values with different superscript in a row differ significantly ($p \leq 0.05$, n=3)

Table 2 Dietary fibers, starch and amylose contents (% dwb) in grains and tubers

	Wheat	Rice	Barley	Bengal gram	Pea	Kidney bean	Lentils	Potato	Sweet potato
IDF	5.4 ± 0.46^{b}	2.1 ± 0.16^{a}	10.7 ± 0.50^{c}	21.6 ± 0.83^{f}	16.7 ± 0.98^{de}	14.9 ± 1.04^{d}	18.0 ± 0.78^{e}	3.9 ± 0.10^{ab}	3.1 ± 0.04^{a}
SDF	2.9 ± 0.30^{cd}	0.9 ± 0.07^{a}	4.3 ± 0.26^{e}	3.3 ± 0.03^{d}	4.7 ± 0.03^{f}	2.3 ± 0.09^{b}	2.8 ± 0.08^{c}	5.5 ± 0.17^{g}	4.4 ± 0.06^{f}
TDF*	8.3	3.0	15.0	24.9	21.4	17.2	20.08	9.4	7.5
Total starch	69.8 ± 1.10^{d}	81.4 ± 1.10^{f}	65.6 ± 0.76^{e}	60.3 ± 1.19^{c}	59.9 ± 1.78^{c}	58.2 ± 0.77^{c}	44.4 ± 0.74^{b}	85.51 ± 1.64^{g}	87.4 ± 1.19^{g}
Amylose (% starch)	25.8 ± 0.66^{e}	20.7 ± 1.14^{b}	23.8 ± 0.54^{cd}	32.3 ± 0.70^{g}	33.6 ± 0.78^{g}	26.8 ± 0.66^{ef}	28.0 ± 0.68^{f}	20.4 ± 0.76^{b}	17.4 ± 0.42^{a}

The values with different superscripts in a row differ significantly ($p \leq 0.05$, n=3) * IDF+ SDF, (Insoluble and soluble dietary fibers)

Table 3 Effect of conventional boiling (H_1) and pressure cooking (H_2) on resistant starch content (% dwb) of some grains and tubers

	Wheat	Rice	Barley	Bengal gram	Pea	Kidney bean	Lentils	Potato	Sweet potato
H_1	1.9 ± 0.21	1.4 ± 0.16	2.8 ± 0.23	4.8 ± 0.10	3.7 ± 0.12	4.3 ± 0.15	5.0 ± 0.11	1.8 ± 0.15	1.4 ± 0.06
H_2	1.8 ± 0.14	1.2 ± 0.08	2.6 ± 0.09	4.6 ± 0.20	3.2 ± 0.11	4.1 ± 0.08	4.9 ± 0.27	1.7 ± 0.08	1.3 ± 0.16

No significant difference between H_1 and H_2 ($p \leq 0.05$)

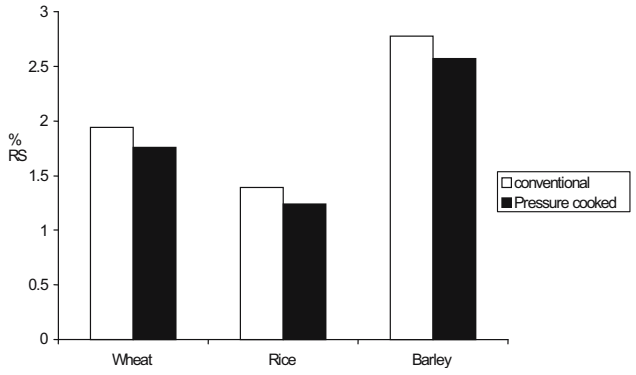

Fig. 1 RS content of cereal flours as affected by heating method

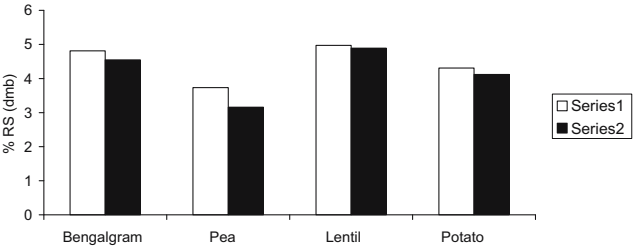

Fig. 2 RS content of conventionally boiled (Series 1) and pressure-cooked (Series 2) legume flours

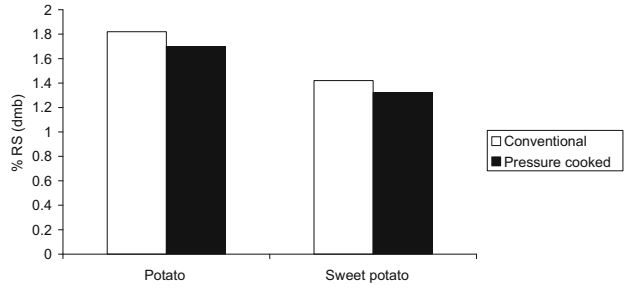

Fig. 3 RS content of potato and sweet potato as affected by heating method

is in line with what could be expected from their polymorph starch type. Cereal starches are 'A' type starches (Gernat et al. 1990, Cairns et al. 1997) always having a low RS fraction of starch. The RS content of H_1 and H_2 tubers was less in comparison to that of legumes and cereals. The two possible reasons for this may be the low amylose content in tuber crops as amylose plays an important role in the formation and development of RS during heat processing and secondly as the tubers contain higher water content, heating results in more uniform gelatinization of starch resulting in to lower amount of RS.

Bishnoi and Khetarpaul (1993) also observed increased starch digestibility in H_2 pea seeds. Unlike our findings, Mahmood et al. (2006) observed an increase in RS content of H_2 tubers. Katyal et al. (2005) also observed an increase in the RS content of H_2 red gram and green gram. The marginal decrease in the RS content of the H_2 foods in comparison to the H_1 counterparts may occur due to more extensive changes in the cell wall integrity during H_2 of foods and this could result in increased accessibility of the starch to amylolytic enzymes. Further, H_2 may facilitate more deep and uniform heat penetration and hence gelatinization of starch granules resulting in increased susceptibility of the starch to amylolytic degradation. H_2 has facilitated alterations in protein/fiber associations, which seems important particularly in legumes.

Conclusion

Legumes contain higher amount of RS in comparison to cereals and tubers. The heating methods affect the content of RS in foods. As pressure-cooking results in more uniform and complete gelatinization of starch and such foods when consumed freshly can be expected to have lesser amounts of RS resulting in more calories. Since, Indian diets involve a large proportion of cereals, legumes and tubers, RS levels can be manipulated by using different heating methods.

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